Date of Approval: May 22, 2009

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-295

PALLADIA

toceranib phosphate
Tablets
dogs

For the treatment of Patnaik grade II or III, recurrent, cutaneous mast cell tumors with or without regional lymph node involvement in dogs.

Sponsored by:

Pharmacia & Upjohn Company, a Division of Pfizer, Inc.

TABLE OF CONTENTS

I. GENERAL INFORMATION:

A. File Number: NADA 141-295

B. Sponsor: Pharmacia & Upjohn Company, a Division of Pfizer Inc
235 East 42d Street, New York, NY 10017
Drug Labeler Code: 000069

C. Proprietary Name(s): PALLADIA

D. Established Name(s): Toceranib phosphate

E. Pharmacological Category: Antineoplastic

F. Dosage Form(s): Tablets

G. Amount of Active Ingredient(s): 10, 15 and 50 mg toceranib (as toceranib phosphate per tablet)

H. How Supplied: PALLADIA tablets are available as round, colored tablets: blue 10 mg tablets, orange 15 mg tablets, and red 50 mg tablets. Each tablet is marked with tablet strength on one side and the Pfizer logo on the other. The tablets are packaged in 30-count bottles.

I. How Dispensed: Rx

J. Dosage(s): Administer an initial dosage of 3.25 mg/kg (1.48 mg/lb) body weight, orally every other day. Dose reductions of 0.5 mg/kg (to a minimum dose of 2.2 mg/kg (1.0 mg/lb) every other day) and dose interruptions (cessation of PALLADIA for up to two weeks) may be utilized, if needed, to manage adverse reactions. Adjust dose based on approximately weekly veterinary assessments for the first 6 weeks and approximately every 6 weeks, thereafter. PALLADIA may be administered with or without food. Do not split tablets.

K. Route(s) of Administration:	Oral
L. Species/Class(es):	Dogs
M. Indication(s):	For the treatment of Patnaik grade II or III, recurrent, cutaneous mast cell tumors with or without regional lymph node involvement in dogs

II. EFFECTIVENESS:

The terms SU11654 and SU011654 represent the free base form and are equivalent terms. PHA-2913639E is the phosphate salt of SU011654. PHA-291639E, toceranib phosphate and PALLADIA are equivalent terms and are used interchangeably throughout this document.

A. Dosage Characterization:

1. Single Center Efficacy and Safety Dose Escalation Study

London CA, Hannah AL, Zadovoskaya R, et al. Phase I dose escalating study of SU11654, a small molecule receptor tyrosine kinase inhibitor, in dogs with spontaneous malignancies. *Clinical Cancer Research* 9(7): 2755 - 2768, 2003.
An open-label, dose escalating study in 57 dogs with spontaneous advanced malignancies that had failed conventional therapies was conducted to assess safety and pharmacokinetics of SU11654. Dose escalation occurred in 1.25 mg/kg increments via 3 separate dosing schedules – once daily, every other day and a 7-day loading dose (dose administered daily for 7 days) followed by alternate daily maintenance therapy - until unacceptable toxicity occurred (severe diarrhea, vomiting, anorexia or neutropenia). The initial starting dose was set at 1.25 mg SU11654/kg. Dogs were treated in 3-week cycles. Dogs were evaluated at a minimum of two 3-week assessments. Dogs receiving daily dosing did not achieve doses above 2.5 mg/kg due to unacceptable toxicity. Dogs receiving every other day dosing achieved doses as high as 3.75 mg/kg, but doses above 3.25 mg/kg produced unacceptable toxicity. Dogs receiving the 7-day loading dose achieved doses as high as 3.75 mg/kg, but doses above 2.5 mg/kg produced unacceptable toxicity. In this study, toxicities resolved in most dogs with temporary discontinuation of therapy for 2-3 days.

Table 1: Adverse reactions reported during the study in each group

Dose mg/kg	No of Dogs	Diarrhea	Anorexia	Vomiting	Fatigue	Hind limb weakness	Neutropenia
1.25 QD	5	40%	80%	40%	0%	40%	20%
2.5 QD	5	60%	80%	40%	0%	40%	0%
1.25 EOD	2	50%	50%	0%	0%	0%	50%
2.5 EOD	16	38%	31%	13%	6%	38%	6%
3.25 EOD	20	50%	40%	5%	20%	15%	0%
3.75 EOD	3	0%	33%	33%	0%	33%	0%
2.5 QD x7; EOD	5	40%	40%	20%	0%	60%	20%
2.75 QDx7; EOD	1	0%	100%	0%	100%	100%	0%

Antihistamines were administered to dogs with mast cell tumors to prevent signs of anaphylaxis, as these tumors are known to release histamine. In addition, to prevent or treat drug-related gastrointestinal toxicities, supportive care, typically consisting of famotidine, metronidazole and metoclopramide, was administered to many of the dogs in the later cohorts. Results showed that every other day dosing resulted in significantly less toxicities and therefore was considered to be the preferred dosing regimen. Serum concentrations of 50 – 100 ng/mL for half of the dosing interval were achieved using 2.5 and 3.25 mg SU11654/kg every other day.

Conclusion: Based on tumor response and safety, a dose of 3.25 mg SU11654/kg every other day was chosen as the starting dose for future studies with the option to reduce the dose in order to maintain a response while minimizing adverse events.

2. Pharmacokinetics

On the basis of preclinical work in rodent models, the therapeutic range of SU011654 (free base form) for target inhibition was considered to be 50-100 ng/mL for 12 hr of a 24-hr dosing period (See London et al. 2003[1]). Four blood level studies in dogs demonstrated that these drug concentrations could be achieved under fed or fasted conditions when dogs are administered doses of 3.25 mg/kg every other day.

a) Study 2002-0142: Laboratory Multiple Dose PK–scored 20 mg oral tablet (target dose 3.25 mg free base equivalents (fbe)/kg; every-other-day dosing). Eleven healthy beagle dogs were administered 2.53 to 3.38 mg fbe/kg doses of PHA-291639E every other day for 2 weeks (7 doses). Food and water were provided ad libitum throughout the study. Plasma was sampled from each dog predose, and 1, 2, 4, 6, 8, 24, 32, and 48 hrs after dose 1, at several times after dose 3 and 5, and at 0, 1, 2, 4, 6, 8, 12, 24, 28, 32, 48, 56, 72, 96, 120, 144, 168, 192, 216 and 240 hrs after the last dose. After dose 1, only 6/11 dogs achieved concentrations $\geq$ 50 ng/mL for 8 hrs postdose, but due to accumulation with repeated administration, 8/11 dogs maintained drug concentrations $\geq$ 50 ng/mL for 8 hrs after dose 7. After dose 7, 2/11 dogs maintained concentrations $\geq$ 50 ng/mL for 24 hrs. After dose 1, only 1 dog achieved concentrations $\geq$ 100 ng/mL. After dose 7, 5/11 dogs had concentrations $\geq$ 100 ng/mL, but this elevated drug concentration occurred for less than 4 hrs in 4/5 of those animals.

b) Study 1963C-60-04-688: Field Multiple Dose PK–scored 20 mg oral tablet (target dose 3.25 mg fbe/kg; every-other-day dosing). This multicenter field study, conducted in client-owned dogs with recurrent mast cell tumors, evaluated every-other-day dosing of PHA-291639E at an initial target dose of 3.25 mg fbe/kg body weight. Pharmacokinetic data were collected for 8 dogs

[1] London CA, Hannah AL, Zadovoskaya R, et al. Phase I Dose-Escalating Study of SU11654, a Small Molecule Receptor Tyrosine Kinase Inhibitor, in Dogs with Spontaneous Malignancies. *Clinical Cancer Research* 9(7):2755-2768; 2003

(3M+5F) after at least three consecutive every-other-day doses following at least 6 weeks of treatment with PHA-291639E. Thus, the plasma concentrations should be at steady-state. Each dog was housed at the trial site or their home and was fed according to the normal feeding pattern for that animal. Dogs were administered 0.934 – 2.99 mg fbe/kg (mean ± SD: 2.56 ± 0.7 mg/kg BW); 1, 2, 3, 5 or 6 tablets (GLP11175 or GMP40,810; 18.8 or 19.9 mg fbe/tablet, respectively; final active pharmaceutical ingredient salt form) based on body weights of 8.2 – 39.4 kg recorded just prior to PK phase (after at least 6 weeks of treatment). The PK phase consisted of three consecutive every-other-day doses given on Days 1, 3, and 5 with blood samples collected on Day 5 at 0 (pre-dose), 1, 2, 4, 6, 8, 12, 24, 28, 32, and 36 hours post-treatment. Plasma concentrations of SU011654 were measured using a non-validated LC-MS/MS method. Drug concentrations at or above 50 ng/mL were achieved in 5/8 client-owned pets. Time to reach concentrations of 50 ng/mL ranged between 2 and 4 hrs. Of the dogs with concentrations $\geq$ 50 ng/mL, this concentration was achieved for a duration no less than 4 hrs. When values are dose-normalized to 3.25 mg/kg, there is still one subject who fails to reach 50 ng/mL and one subject who barely exceeds 50 ng/mL. However, 2/8 subjects have plasma concentrations that exceed 100 ng/mL.

c) Study 2003-0099: Fed/Fasted PK – Scored 20 mg oral tablet (target dose 3.25 mg fbe/kg). Beagle dogs (6M+6F) were administered PHA-291639E (phosphate salt) at 2.88-3.84 mg (mean ± SD: 3.30 ± 0.31 mg) fbe/kg (target 3.25 mg fbe/kg; 1.5, 2, or 2.5 tablets, non-final formulation). In each dose phase, half the dogs (3M+3F) were fed 100-150 mL of food/water slurry one hour prior to dose as a portion of their daily ration. These dogs then had immediate ad libitum access to the remainder of their daily ration. The remaining dogs were fasted for at least 12 hours prior to dose through 8 hours after dose. Food was provided to fed and fasted groups approximately 8 hours after dosing. Plasma samples were obtained from each dog at 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 28, 32, 48, 56, and 72 hours after each dose. Plasma concentrations of SU011654 were measured using a non-validated LC-MS/MS analytical method. In this study, all dogs had drug concentrations exceeding 50 ng/mL for at least 8 hrs, with 3/12 dogs having values exceeding the 50 ng/mL target for 24 hrs. Seven of 12 subjects also achieved drug concentrations at or above 100 ng/mL. Food was found to not significantly influence tablet bioavailability.

d) Study 1560N-60-06-756: Bioavailability and PK. Eight dogs (4 males/4 females) were dosed with PHA-291639E, either as oral tablets (final formulation phosphate salt) at 3.25 mg/kg or intravenous solution at 1.00 mg/kg. After dosing PHA-291639E orally at 3.25 mg/kg, the mean (± 1SD) C_{max} of PHA-291639 was 68.6 (± 12.9) ng/mL. C_{max} was observed at 7.0 hr after dosing. The terminal half-life (harmonic mean), $t_{1/2}$, was 16.8 hr (range

13.7 to 19.1 h). Absolute oral bioavailability was estimated to be 76.9% with a 95% CI of 57.2% to 103.5%. After IV dosing of PHA-291639E at a targeted dose of 1.0 mg/kg, $t_{1/2}$ was 17.5 hr, approximately the same as after oral dosing, indicating that the elimination is not limited by absorption. Clearance was moderate at 1.45 L/hr/kg. The apparent volume of distribution, Vss, was 29.7 L/kg indicating the drug is well distributed and probably sequestered in various tissues.

3. Pharmacodynamics (Target Modulation)

A study by Pryer, et al, (Pryer NK, Lee LB, Zadovoskaya R, Yu X, et al. Proof of target for SU11654: inhibition of KIT phosphorylation in canine mast cell tumors. *Clinical Cancer Research* 9(15):5729 - 5734; 2003) determined the effect of SU11654 on KIT (stem cell factor receptor) in canine mast cell tumors and plasma drug concentrations in dogs with advanced mast cell tumors. Tumor biopsies were obtained from 14 dogs before and 8 hours after administration of a single oral target dose of 3.25 mg SU11654/kg body weight. Drug was administered orally as tablets. The mean plasma concentration at 8 hours post-dosing was 105 ng/mL (SD, ± 9 ng/mL). Of the 11 tumor samples evaluable for KIT target modulation, 8 showed reduced levels of phosphorylated KIT relative to total KIT after treatment with SU11654 compared to pretreatment biopsies.

B. Substantial Evidence:

1. Field Study

A single field study, comprised of a 6-week masked phase followed by an open-label phase, was conducted to support substantial evidence of effectiveness and safety for the treatment of mast cell tumors in dogs.

a) Study Title and Number: Multicenter, Placebo-Controlled, Double-Blind, Randomized Study of Oral PHA-291639E in the Treatment of Dogs with Recurrent Mast Cell Tumors. Study #1963C-60-04-688.

b) Type of Study: GCP, Field safety and effectiveness study

c) Study Dates: February 13, 2003 to August 25, 2005

d) Location(s) and Investigator(s):

Name	City	State
Dr. Philip Bergman	New York	New York
Dr. Guillermo Couto	Columbus	Ohio
Dr. Carolyn Henry	Columbia	Missouri
Dr. John Hintermeister	Buffalo Grove	Illinois
Dr. Chand Khanna	Washington, DC	-
Dr. Mary Kay Klein	Tucson	Arizona
Dr. Cheryl London	Davis	California
Dr. Neal Mauldin	Baton Rouge	Louisiana
Dr. Kathy Mitchener	Memphis	Tennessee
Dr. Mona Rosenberg	Tustin	California

e) General Design

Purpose of Study: To evaluate the effectiveness and safety of PHA-291639E in the treatment of mast cell tumors in client-owned animals that had recurrent measurable disease after surgery. To determine if objective responses (complete or partial response) were achieved with PHA-291639E treatment in dogs with mast cell tumors compared to placebo treatment. This objective was evaluated by analysis of the response rates at the end of the 6-week masked phase.

Description of Test Animals: One hundred and fifty-one client-owned dogs with Patnaik grade II or III, recurrent, cutaneous mast cell tumors with or without regional lymph node involvement were initially enrolled in the study. Dogs may have had a limit of one completed radiation protocol and a limit of one prior systemic chemotherapy regimen. The most common breed was mixed breed followed by Labrador Retriever, Golden Retriever, Boxer, and Cocker Spaniel. Dogs ranged in age from 3 to 15 years old with the average around 9 years old. Table 2 shows the number of dogs in each group with Grade I, II or III mast cell tumors with or without lymph node involvement.

Table 2: Number of dogs with mast cell tumors, with or without lymph node involvement

Tumor Grade/Lymph Node Involvement	# of Dogs	
	Placebo	Treated
I –No	1	0
I – Yes	1	0
II – No	27	44
II – Yes	14	25
III – No	10	8
III – Yes	10	9
Total	**63**	**86**

Control and Treatment Group(s): Table 3 shows the number of dogs enrolled in each treatment group, and the average age, weight and gender for each group.

Table 3: Number of dogs enrolled in each treatment group in the initial 6-week masked phase

Group	# of Dogs	Average Age (years)	Average Weight (kg)	Gender
Treated	87	8.7 (3.1 – 15.3)	28.8 (5.4 – 68.9)	51 F (0 intact), 36 M (1 intact)
Placebo*	64	9.2 (4.0 – 14.6)	31.1 (5.7 – 64.8)	38 F (1 intact), 26 M (4 intact)

*Placebo control was the tablet minus active ingredients.

One hundred and forty-nine dogs (86 treated, 63 control) were included in the masked phase effectiveness analysis. Two dogs (one from each treatment group) were removed from the effectiveness analysis but were included in the safety analysis.

One hundred and forty-five dogs were included in the masked plus extended therapy phase.

Randomization: Dogs were randomized at a ratio of 4:3 (PHA-291639E:Placebo). Dogs were blocked based on histopathologic tumor grade (Patnaik grade II or III) and tumor burden (regional lymph node positive or negative for mast cell tumor).

Masking: Treatment groups were masked to all investigator personnel and animal owners.

Inclusion and Exclusion Criteria:

- Inclusion Criteria:
  - Dogs must have been diagnosed with Patnaik grade II or III, recurrent, cutaneous mast cell tumor with or without regional lymph node involvement.
  - Dogs must have been at least 1 year of age and a minimum of 5 kg.
  - Dogs must have had adequate organ function as indicated by standard laboratory tests.
  - Dogs must have had a Performance Status[2] score of 0 or 1.

[2] The Performance Status Scale is a 5-point scale used to subjectively assess a patient's daily performance. A score of 0 corresponds to "Normal; full active; able to perform at pre-disease level" and a score of 1 corresponds to "Restricted; restricted activity from pre-disease level, but able to function as an acceptable pet."

  - Owners must have understood and signed an informed consent document.

- Exclusion Criteria:
  - Evidence of gastrointestinal bleeding due to mast cell disease.
  - Any serious systemic disorder incompatible with the study.
  - Any male or female dog used for breeding purposes, including gestating bitches.
  - Systemic mast cell tumor or involvement of more than one lymph node region.

Drug Administration: PHA-291639E was initially administered at 3.25 mg/kg body weight orally, every other day. PHA-291639E was administered with or without food.

Dose Modification: Dose reductions to a minimum of 2.2 mg PHA-291639E/kg every other day and dose interruptions (cessation of PHA-291639E for up to 2 weeks) were utilized, as needed, to manage adverse events. Dose was adjusted based on approximately weekly veterinary assessments for the first 6 weeks and approximately every 6 weeks, thereafter.

Dose Reductions: One prescribed dose reduction was made for 18.4% (16/87) of treated dogs compared to 6.3% (4/64) of placebo dogs during the masked phase. One treated dog had 2 dose reductions. Dose reductions within the treated group occurred most commonly in dogs with Grade III mast cell tumors 35.3% (6/17).

For the masked and extended therapy phase, 21.4% (31/145) had 1 dose reduction, 9.0% (13/145) had 2 dose reductions and 1 dog (0.7%) had 3 dose reductions. Dose reductions occurred most commonly in dogs with Grade II mast cell tumors 33.3% (36/108).

Drug Interruptions (cessation of drug for up to 2 weeks): One prescribed drug interruption was made for 40.2% (35/87) of treated dogs compared to 9.4% (6/64) of placebo dogs during the masked phase. 6.9% (6/87) of treated dogs had 2 drug interruptions and 1.6% (1/64) of placebo dogs had 2 drug interruptions. 1.1% (1/87) of treated dogs had 3 drug interruptions and no placebo dogs had more than 2 drug interruptions. Within the treated dogs, more drug interruptions occurred in dogs with Grade III mast cell tumors 58.8% (10/17) compared to dogs with Grade II mast cell tumors 45.7% (32/70).

For the masked and extended therapy phase, 15.9% (23/145) of the dogs did not have a drug interruption. 53.8% (78/145) had 1 drug interruption, 15.9% (23/145) had 2 drug interruptions, 11.0% (16/145) had 3 drug interruptions and approximately 3% (5/145) had 4 or more drug interruptions.

Variables Measured: Objective response rate, juxtamembrane (JM) c-*kit* mutation status of tumor, clinical pathology, and adverse events.

Objective tumor response: tumor response was evaluated at 3 and 6 weeks during the masked phase according to the Response Evaluation Criteria in Solid Tumors (RECIST) Guidelines adapted for canine mast cell tumors shown below in Table 4 and 5.

Table 4: General disease response definitions:

Response	**Definition**
Complete Response (CR)	Disappearance of all target lesions. Non-target lesions: Disappearance of all non-target lesions (and appearance of no new lesions).
Partial Response (PR)	At least 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD. Non-target lesions: Not applicable.
Progressive Disease (PD)	At least 20% increase in the sum of the LD target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions. Non-target lesions: unequivocal progression of non-target lesions, in the judgment of the evaluator, or the appearance of one or more new lesions was considered to be PD regardless of target lesion response assessment.
Stable Disease (SD)	Absence of criteria for either a response or progression of target lesions. Non-target lesions: Not applicable.

Table 5: Overall response

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR or None	No	Complete Response
CR	Non-CR/Non-PD	No	Partial Response
PR	Non-PD	No	
SD	Non- PD	No	Stable Disease
PD	CR, PD, Non-CR/ Non-PD, None	Yes or No	Progressive Disease
CR, PR, SD, PD	PD	Yes or No	
CR, PR, SD, PD	CR, PD, Non-CR/ Non-PD, None	Yes	

Statistical Analysis: The primary effectiveness variable was the objective response (complete + partial response as defined in Table 4) at the end of the masked phase (Week 6). The objective response rates between the PHA-291639E and placebo groups were compared using logistic regression analysis. In addition to treatment effect, the statistical model included the fixed effects of tumor burden, tumor grade, JM c-*kit* mutation, and all two-way interaction terms between treatment and each fixed effect.

f) Results: There was a statistically significant improvement in objective response (OR) for PHA-291639E treatment compared to placebo treatment ($P < 0.001$) as shown in Table 6. The difference in OR rate between groups was not significantly associated with tumor grade (Grade II or III) or tumor burden (presence vs. absence of regional lymph node involvement) ($P > 0.05$), see Table 7.

Table 6: Mast Cell Tumor – primary effectiveness endpoint

Effectiveness Parameter	Placebo (n = 63)	PHA-291639E (n = 86)	P-value
Objective Response Rate*	7.9% (5/63)	37.2% (32/86)	< 0.001

*Objective response is complete response (CR) + partial response (PR)

Table 7: Objective Response (OR)* Rates for covariates tumor burden and central pathology tumor grade

	Placebo			Treated		
	# of dogs in group	**# with OR**	**%**	**# of dogs in group**	**# with OR**	**%**
Overall	63	5	7.9%	86	32	37.2%
Lymph Node Involved						
No	38	4	10.5%	52	22	42.3%
Yes	25	1	4%	34	10	29.4%
Tumor Grade						
I	2	0	0%	0		
II	41	5	12.2%	69	25	36.2%
III	20	0	0%	17	7	41.2%
Lymph Node & Tumor Grade						
No – I	1	0	0%	0		
No – II	27	4	14.8%	44	19	43.2%
No – III	10	0	0%	8	3	37.5%
Yes – I	1	0	0%	0		
Yes – II	14	1	7.1%	25	6	24.0%
Yes – III	10	0	0%	9	4	44.4%

*Objective response is complete response (CR) + partial response (PR)

Table 8 shows the objective tumor response results within each group at the end of the 6 week masked phase of the study. 8.1% of the treated dogs and none of the placebo dogs were able to achieve a complete response. A few dogs discontinued participation in the study due to adverse events or the owner's request.

Table 8: The number of dogs in each group with a Complete Response, Partial Response, Stable Disease, Progressive Disease, and dogs that discontinued (D/C) participation in the study due to adverse events or the owner's request

Response Rate	Placebo		Treated	
	N	%	N	%
Complete Response (CR)	0	0%	7	8.1%
Partial Response (PR)	5	7.9%	25	29.1%
Stable Disease (SD)	14	22.2%	18	20.9%
Progressive Disease (PD)	40	63.5%	28	32.6%
D/C for Adverse Event[1]	1	1.6%	3	3.5%
D/C due to owner request[2]	3	4.8%	5	5.8%
Total	63	100%	86	100%

[1] One placebo dog had stable disease (SD) and one treated dog had progressive disease (PD).

[2] Two placebo dogs had progressive disease (PD) and two treated dogs had stable disease (SD) and one had progressive disease (PD).

JM c-kit mutation

Mast cell tumors positive for the JM c-kit mutation have a higher objective response compared to those negative for the JM c-kit mutation, however this was not statistically significant.

Table 9: Objective Response (OR) Rates for covariate JM c-kit mutation status

	Placebo			Treated		
	# of dogs in group	# with OR	%	# of dogs in group	# with OR	%
Overall	63	5	7.9%	86	32	37.2%
c-kit Mutation						
No Sample	0	0	0%	2	0%	0%
Negative	54	4	7.4%	64	20	31.3%
Positive	9	1	11.1%	20	12	60.0%

Safety

The safety analysis included 151 animals (64 placebo-treated, 87 PHA-291639E-treated). The National Cancer Institute's Canine-Adapted Common Toxicity Criteria (C-ACTC) was used to assign severity of grade 1, 2, 3 or 4 to the clinical signs. For those adverse events not listed in Table 10, grades 1, 2, 3 and 4 corresponded to mild, moderate, severe and life-threatening, respectively.

Table 10: Canine Adapted Common Toxicity Criteria

Toxicity	Grade	Criteria
Anorexia	**0**	Normal
	1	Inappetence
	2	Anorexia <3 days
	3	Anorexia 3-5 days
	4	Anorexia >5 days, 10% weight loss
Vomiting	**0**	Normal
	1	Nausea (ptyalism, retching, lip licking)
	2	sporadic, self-limited vomiting
	3	<5 episodes per day, <2 days
	4	>5 episodes per day, >2 days; requires hospitalization
Diarrhea	**0**	Normal
	1	Soft stools, responds to dietary modification
	2	<4 watery stools per day, <2 days
	3	4-7 watery stools per day, or >2 days
	4	>7 watery stools per day or bloody; requires hospitalization
GI Bleeding	**0**	Normal
	1	Sporadic fresh blood flecks, sporadic black stool
	2	Black tarry stool or fresh blood in stool >2 days
	3	Frank hemorrhage/blood clots in stool; frank hemorrhage in oral cavity
	4	Catastrophic bleeding, requiring major non-elective intervention
Packed Cell Volume (Hematocrit)	**0**	30-55%
	1	26-30%
	2	22-26%
	3	18-22%
	4	<18%
Neutrophil Count	**0**	Neutrophils 3000-11500/µl
	1	Neutrophils 1500-3000/µl
	2	Neutrophils 1000-1500/µl
	3	Neutrophils 500-1000/µl
	4	Neutrophils <500/µl
Platelet Count	**0**	Platelets 200,000-500,000/µl
	1	Platelets 100,000-200,000/µl
	2	Platelets 50,000-100,000/µl
	3	Platelets 15,000-50,000/µl
	4	Platelets <15,000/µl
Creatinine	**0**	Creatinine 0.5-1.6 mg/dl
	1	Creatinine 1.7-2.0 mg/dl
	2	Creatinine 2.0-3.5 mg/dl
	3	Creatinine 3.5- 4.5 mg/dl
	4	Creatinine >4.5 mg/dl or clinical uremia
Limb Weakness/ Stiffness/ Pain	**0**	Normal gait
	1	Abnormal gait/lame while walking
	2	Protects limb from weight-bearing
	3	No weight bearing on affected limb
	4	Prostrate
Alanine Amino-transferase (ALT)	**0**	≤1X UNL
	1	IU/l 1X-3X UNL
	2	IU/l 3X-4X UNL
	3	IU/l 4X-6X UNL
	4	>6X UNL
Albumin	**0**	2.5-4.2 g/dl
	1	2.0-2.5 g/dl
	2	1.5-2.0 g/dl
	3	1.0-1.5 g/dl
	4	<1.0 g/dl
Bilirubin	**0**	<1.0 mg/dl
	1	1.0-2.0 mg/dl
	2	2.0-3.0 mg/dl
	3	3.0-4.0 mg/dl
	4	>4.0 mg/dl

Hematology

RBC: More treated dogs compared to placebo dogs had decreases in hematocrit, though this was not considered statistically significant. Three dogs treated with drug developed severe anemia (grade 3 or 4) while no placebo dogs developed severe anemia.

Leukocytes (WBC): Significantly more treated dogs compared to placebo dogs had decreases in WBCs from within or above the reference range at baseline to below the reference range during the masked phase ($P < 0.0001$). This was typically characterized as a neutropenia.

Neutrophils: Significantly more dogs in the treated group (46%) compared to the placebo group (6.3%) had a neutropenia of C-ACTC-defined severity grade 1 or 2 ($P < 0.0001$). No dogs had a neutropenia of C-ACTC –defined severity grade 3 or 4 during the masked phase. During the extended phase, 9 dogs or 6.2% had neutrophil counts that continued to decline throughout the study while receiving drug. Two of these dogs developed a grade 3 neutropenia that required a drug interruption while 7 of these dogs maintained a grade 2 neutropenia.

Monocytes: Significantly more placebo dogs compared to treated dogs had increases in monocytes during the masked phase ($P \leq 0.05$).

Eosinophils: Significantly more treated dogs compared to placebo dogs had decreases in eosinophils during the masked phase ($P = 0.0001$).

Platelets: Both the placebo (20.3%) and the treated (24.1%) dogs had thrombocytopenia of C-ACTC-defined severity of grade 1 or 2 during the masked phase of the study; no dogs had grade 3 or 4 thrombocytopenia. 28.3% of dogs had thrombocytopenia in the masked plus extended therapy phase. Of these, 2.1% of dogs had a C-ACTC-defined severity of grade 3 or 4 during the extended therapy phase.

There were 9 cases of concurrent decreased neutrophils and platelets. Only 1 case had a grade 2 or higher for both variables concurrently.

Prolonged Partial Thromboplastin Time (PTT): Five dogs treated with drug and no placebo dogs had clinically relevant prolongations of PTT in the masked and extended therapy phase. Prothrombin Time (PT) was normal in these dogs therefore the intrinsic pathway was affected, which includes factors XII, XI, and VIII.

Serum Chemistry

Albumin: More treated dogs (12.6%) had hypoalbuminemia of C-ACTC-defined severity 1 or 2 compared to placebo dogs (7.8%) during the masked phase of the study. Overall, during the masked plus extended therapy phase, 12 treated dogs had albumin levels less than 2.0 g/dL; of these 2 cases had grade 3 or 4 hypoalbuminemia. Four of these cases died; these deaths were possibly drug related. See the discussion below on deaths for more details. Three dogs had a continual trend of decreasing albumin throughout the study.

Several dogs developed elevated BUN and/or elevated creatinine during the study. The prestudy blood work was normal for these dogs.

Several dogs that required a drug interruption developed at least one of the following: azotemia (elevated BUN and creatinine), hypoalbuminemia, hyperphosphatemia and anemia. These trends were not statistically significant. Four dogs died with these clinical pathology abnormalities. See the discussion below on deaths for more details.

Alanine aminotransferase (ALT): An increase in ALT was seen in both the placebo dogs and the treated dogs with about the same frequency. The increase is most likely disease related and not drug related.

Bilirubin: More treated dogs (5.7%) had hyperbilirubinemia compared to placebo dogs (1.6%) during the masked phase of the study. Overall, during the masked plus extended therapy phase, 6.9% of dogs had hyperbilirubinemia.

Urinalysis

The development of a urinary tract infection (hematuria, proteinuria, and active sediment) was seen more frequently in treated dogs than placebo dogs.

Tables 11 and 12 summarize the abnormal clinical pathology variables that occurred at >5% frequency in the dogs during the masked and extended therapy phase.

Table 11: Frequency of all C-ACTC-defined clinical pathology-related toxicities (any grade)

Clinical Pathology Variable	Placebo Dogs (n = 64)	Treated Dogs (n = 87)	Masked plus extended phase (n = 145)
Neutropenia	6.3%	46%	44.8%
ALT increased	21.9%	24.1%	27.6%
Thrombocytopenia	20.3%	24.1%	28.3%
Hematocrit decreased	7.8%	5.7%	11.0%
Hypoalbuminemia	7.8%	12.6%	28.3%
Creatinine increased	4.7%	5.7%	13.8%
Hyperbilirubinemia	1.6%	5.7%	6.9%
Urinary Tract Infection	1.6%	5.7%	7.6%

Table 12: Masked phase: Frequency of all C-ACTC-defined clinical pathology-related toxicities (grade 3 or 4)

Clinical Pathology Variable	Placebo Dogs (n = 64)	Treated Dogs (n = 87)	Masked plus extended phase (n = 145)
Hematocrit decreased	0.0%	3.4%	2.8%
ALT increased	4.7%	1.1%	4.1%
Hyperbilirubinemia	1.6%	0.0%	0.0%
Thrombocytopenia	0.0%	0.0%	2.1%
Hypoalbuminemia	0.0%	0.0%	1.4%
Creatinine increased	0.0%	0.0%	1.4%
Neutropenia	0.0%	0.0%	1.4%

Body Weight

There is a drug related effect on body weight. 20.0% of dogs >13% weight loss in the masked plus extended phase attributable to the drug. Five of these dogs had >25% weight loss.

Adverse Reactions

The most common adverse reactions (≥ 5% of PHA-291639E -treated animals) during the 6-week masked phase are summarized in the Table 12; those reported during the entire study (masked phase combined with the open label phase) are summarized in Table 13. There was no significant difference between groups in the number of animals experiencing grade 3 or 4 (out of 4) adverse reactions or in the number of animals discontinued from study due to adverse reactions.

Table 13: Summary of the most common adverse reactions during the masked phase

	Placebo (n = 64)		PHA-291639E (n = 87)	
Adverse Reaction	**Any Grade[1]**	**Grade 3 or 4**	**Any Grade[1]**	**Grade 3 or 4**
Diarrhea	26.6% [2]	3.1%	46.0% [2]	6.9%
Anorexia	31.3%	6.3%	39.1%	6.9%
Lethargy	29.7%	3.1%	35.6%	4.6%
Vomiting	32.8%	6.3%	32.2%	9.2%
Lameness	9.4%	0.0%	17.2%	0.0%
Weight loss	3.1% [2]	0.0%	14.9% [2]	1.1%
Musculoskeletal disorder	6.3%	0.0%	11.5%	1.1%
Blood in stool/GI bleed/hemorrhagic diarrhea	3.1% [2]	0.0%	12.6% [2]	2.3%
Dehydration	4.7%	0.0%	9.2%	2.3%
Dermatitis	9.4%	1.6%	9.2%	0.0%
Pruritus	4.7%	0.0%	9.2%	0.0%
Tachypnea	4.7%	0.0%	8.0%	1.1%
Localized pain	4.7%	0.0%	8.0%	0.0%
Nausea	3.1%	0.0%	8.0%	1.1%
General pain	4.7%	1.6%	6.9%	0.0%
Polydipsia	7.8%	0.0%	6.9%	0.0%
Pyrexia	3.1%	0.0%	5.7%	2.3%
Flatulence	3.1%	0.0%	5.7%	0.0%
Pigmentation disorder	1.6%	0.0%	5.7%	0.0%
Laboratory Abnormality	**Any Grade[1]**	**Grade 3 or 4[1]**	**Any Grade[1]**	**Grade 3 or 4[1]**
Neutropenia	6.3% [2]	0.0%	46.0% [2]	0.0%
Thrombocytopenia	20.3%	0.0%	24.1%	0.0%
Increased ALT	21.9%	4.7%	24.1%	1.1%
Hypoalbuminemia	7.8%	0.0%	12.6%	0.0%
Hyperbilirubinemia	1.6%	1.6%	5.7%	0.0%
Decreased Hematocrit	7.8%	0.0%	5.7%	3.4%
Increased Creatinine	4.7%	0.0%	5.7%	0.0%
Urinary tract infection	1.6%	0.0%	5.7%	0.0%

[1] Grading of laboratory abnormalities was based on the National Cancer Institute's Common Toxicity Criteria guideline adapted for canines (Canine-Adapted Common Toxicity Criteria).

[2] Occurred in significantly ($P \leq 0.05$) more PHA-291639E-treated than placebo-treated dogs. Only the first observation of an adverse reaction in any given dog was included in the occurrence calculation. Other adverse events were reported but occurred in < 5% of PHA-291639E-treated dogs. Any individual dog may have had multiple adverse reactions.

Table 14: Summary of the most common adverse reactions during the study (masked phase combined with the open-label phase)

	PHA-291639E (n = 145) [1]	
Adverse Reaction	**Any Grade**	**Grade 3 or 4**
Diarrhea	58.6%	8.3%
Anorexia	49.7%	8.3%
Vomiting	47.6%	9.7%
Lethargy	39.3%	4.1%
Lameness	22.8%	0.0%
Weight loss	21.4%	2.8%
Blood in stool/GI Bleed/Hemorrhagic diarrhea	18.6%	2.8%
Dehydration	15.2%	2.1%
Pruritus	12.4%	0.0%
Pigmentation disorder	11.7%	0.0%
Dermatitis	11.0%	0.0%
Musculoskeletal disorder	11.0%	0.0%
General pain	8.3%	0.0%
Otitis externa	8.3%	0.0%
Tachypnea	8.3%	0.0%
Nausea	7.6%	1.4%
Polydipsia	7.6%	0.0%
Pyrexia	6.9%	2.8%
Arthritis	6.2%	0.0%
Localized edema	6.2%	0.0%
Bacterial skin infection	5.5%	0.0%
Conjunctivitis	5.5%	0.0%
Laboratory Abnormality	**Any Grade[2]**	**Grade 3 or 4[2]**
Neutropenia	44.8%	1.4%
Hypoalbuminemia	28.3%	1.4%
Thrombocytopenia	28.3%	2.1%
Increased ALT	27.6%	4.1%
Increased Creatinine	13.8%	1.4%
Decreased Hematocrit	11.0%	2.8%
Hyperbilirubinemia	6.9%	0.0%
Urinary tract infection	7.6%	0.0%

[1] All dogs received at least 1 dose of PHA-291639E.
[2] Grading of laboratory abnormalities was based on the National Cancer Institute's Common Toxicity

Criteria guideline adapted for canines (Canine-Adapted Common Toxicity Criteria).
Only the first observation of an adverse reaction in any given dog was included in the occurrence calculation. Other adverse reactions were reported but occurred in < 5% of dogs. Any individual dog may have had multiple adverse reactions.

Lameness/Musculoskeletal disorder: A musculoskeletal disorder was reported in 32.4% dogs that received at least 1 dose of drug during the masked plus extended therapy phase. Many dogs developed these clinical signs after being on drug for only 3 to 6 weeks. Most resolved with either no treatment or concomitant use of standard of care.

Table 15: Frequency of occurrence of musculoskeletal disorder adverse reactions (any grade) compared to frequency of occurrence of musculoskeletal disorder adverse reactions (any grade) attributed to the drug or unknown

Adverse Reaction	Placebo Dogs n = 64	Treated Dogs n = 87	Masked plus Extended Phase n =145
Any Musculoskeletal disorders (SOC)[1]	15.6%	27.6%	32.4%
Lameness	9.4%	17.2%	22.8%
Musculoskeletal Disorder (NOS)[2]	6.3%	11.5%	11.0%
Arthritis	1.6%	3.4%	6.2%
Bone and joint disorder	0.0%	1.1%	1.4%
Joint pain	0.0%	1.1%	1.4%
Musculoskeletal Neoplasm	0.0%	1.1%	0.7%
Disc prolapse	0.0%	0.0%	0.7%
Muscle pain	0.0%	0.0%	0.7%
Muscle stiffness	0.0%	0.0%	0.7%
Myopathy	0.0%	0.0%	0.7%
Myositis	0.0%	0.0%	0.7%

[1] SOC – system organ class as classified by Veterinary Medicinal Dictionary for Drug Regulatory Agencies (VEDDRA)
[2] NOS – not otherwise specified as classified by VEDDRA

Pigmentation Disorders/Alopecia: Seven dogs developed nasal depigmentation with the first few weeks of treatment. Eleven dogs developed color changes to their haircoat or skin after being on drug for a longer period of time, typically 18-72 weeks on drug. Two dogs completely changed their haircoat. A Rhodesian ridgeback changed to white and a Golden Retriever changed from deep red to blonde. Three dogs developed pigmentation; one was described as melanosis. Seven dogs developed patchy alopecia.

Epistaxis: Three dogs had epistaxis. One dog had bleeding from the nose and mouth and bleeding from the third eye lid from days 48 to 51 after starting drug. The dog's platelets were mildly elevated 7 days prior to this event at 499 thousand/mcL (170 – 400 thousand/mcL) and 14 days afterward at >600 thousand/mcL. The second dog had epistaxis on days 87 – 94 after starting drug. The dog's platelets were low 11 days prior to the event, 145 thousand/mcL (164 – 510 thousand/mcL). The decrease was not considered clinically relevant. One month after the event, platelets were within the normal range. The third dog developed epistaxis and disseminated intravascular coagulation 56 days after starting drug. The platelet count was decreasing from within normal limits to 45,000/mcL.

Gastrointestinal ulceration/perforation: During the masked phase of the study, 12.6% treated dogs had blood in the stool, gastrointestinal bleeding or hemorrhagic diarrhea compared to 3.1% placebo treated dogs. During the masked plus extended therapy phase of the study, 18.6% dogs had blood in the stool, gastrointestinal bleeding or hemorrhagic diarrhea with 2.8% dogs having severe bleeding classified as C-ACTC-defined grade 3 or 4. One dog was diagnosed with gastric ulceration, which was possibly drug related. Three dogs had gastric (1 dog) or duodenal (2 dogs) perforations during the study. One dog died after completing the placebo phase of the study and had taken 1 dose of study drug. One dog died after receiving drug for 221 days. See a discussion of the dog below.

Seizures/Collapse: Three dogs had seizure like activity while on study. It can not be determined if these were drug related.

Death: There were 5 deaths that were possibly drug related. Pathology findings generally revealed evidence of vascular dysfunction including pulmonary thromboembolism (post-operative); multi-organ failure associated with vasculitis and thrombosis; vascular thrombosis with disseminated intravascular coagulopathy (DIC) and pancreatitis; and vasculitis with DIC. One dog died secondary to gastric perforation; tumor response in this dog was complete response and the duration of treatment with PHA- 291639E was 221 days.

The relation of the following deaths due to drug are unknown. One dog, first treated for 3 weeks with a placebo, died of unknown cause 7 days after initiation of treatment with the drug; no necropsy was conducted. Another dog died of unknown cause 92 days after initiation of treatment with the drug; no necropsy was conducted.

Concomitant Treatments: The following concomitant medications listed in decreasing frequency, were administered to both groups (≥ 5% of dogs) in the masked phase at about equal frequency: diphenydramine, famotidine, metoclopramide, fluid with electrolytes, amoxicillin, clavulanate potassium, enrofloxacin, sucralfate, omeprazole, carprofen, cimetidine, dolasetron mesylate, loperamide and ranitidine. More dogs in the treated group received metronidazole compared to the control group. Additional concomitant medications administered in the masked plus extended therapy phase were: cephalexin and ampicillin.

g) Conclusion: When evaluated at a starting dose of 3.25 mg/kg every other day for 6 weeks, PHA- 291639E was 37.2% effective for the treatment of recurrent Patnaik grade II or III cutaneous mast cell tumors with or without lymph node involvement in dogs. Dose reductions to as low as 2.2 mg/kg body weight and/or drug interruptions were used as needed to manage adverse reactions. The most frequent adverse reactions (>15%) were diarrhea, anorexia, lethargy, vomiting, lameness, weight loss, blood in stool (including gastrointestinal bleeding or hemorrhagic diarrhea), dehydration, neutropenia (grade 1 – 2), hypoalbuminemia (grade 1 – 2), and thrombocytopenia (grade 1 – 2).

III. TARGET ANIMAL SAFETY:

A. Margin of Safety Study

a) Study Title and Number: PHA-291639E: 13-week Oral Margin-of-Safety Study in Dogs, Study #0258-2006

b) Type of Study: GLP laboratory study

c) Study Dates: August 28 – December 21, 2006

d) Location and Investigator:

Nerviano Medical Sciences S.r.l.
Nerviano, Italy
Study Director: Guido Di Gallo

e) General Design

Purpose of Study: The purpose of this study was to demonstrate the safety of PHA-291639E administered orally once every other day to dogs at 0, 2.0, 4.0, and 6.0 mg/kg (0, 0.5, 1, or 1.5 times the clinical dose), for 13 weeks.

Description of Test Animals: Forty Beagle dogs (20 males and 20 females), 24-27 months old, body weight between 6.22 and 15.11 kg before first dosing.

Control and Treatment Group(s):

Table 16: Treatment and Control Groups and Number of Dogs per Group

Tx Group	Dose	Number and Sex of Animals
T01	0 mg/kg (0X)	12 (6M, 6F)
T02	2.0 mg/kg (0.5X)	8 (4M, 4F)
T03	4.0 mg/kg (1X)	12 (6M, 6F)
T04	6.0 mg/kg (1.5X)	8 (4M, 4F)

Randomization: The dogs were randomly assigned using a split plot design with sex as the whole plot factor and treatment as the split plot factor. Within whole plots, the 20 eligible dogs were divided into 4 blocks (2 blocks of size 6 and 2 blocks of size 4) by similarity of body weight. Dogs in each block of 4 (or 6) were individually housed in adjacent pens. The treatments were randomly allocated to dogs in each block; one dog per treatment in the blocks of size 4, and T01 and T03 were assigned 2 dogs each in the blocks of size 6.

Drug Administration: PHA-291639E was administered orally at 0, 2.0, 4.0 or 6.0 mg/kg once every other day 1 hour after eating, for 13 weeks. Placebo (containing excipients only) was administered as a tablet combination equivalent to the 6 mg/kg tablet combination. If a dog vomited or regurgitated a tablet, re-administration was not performed.

Variables Measured: Survival and moribundity, clinical observations, general health observations, physical examination, body weights, food consumption, ophthalmoscopic examinations, electrocardiograms, clinical pathology, necropsy and histopathology.

Statistical Methods: For all analyses, the individual dog was treated as the experimental unit. Variables measured at more than one timepoint, such as body weight, were analyzed using a mixed linear model for repeated measures. The model included fixed effects of a baseline covariate, sex, treatment, sex by treatment interaction, time, sex by time interaction, treatment by time interaction, and the three-way interaction of sex, treatment, and time. Random effects included block within sex, between animal error and within animal error. The correlation structure among the repeated measures was estimated using either the spatial power or ARIMA(1) structures. For variables measured once, such as organ weights, the mixed linear

model included fixed effects of sex, treatment and sex by treatment interaction. Random effects included block and between animal error. Follow-up pairwise mean comparisons between the control group and the treated groups were performed, as necessary, using linear contrasts with significance level 0.10.

f) Results

Two dogs (one male and one female) in the 6 mg/kg group were euthanized for treatment-related clinical toxicities. Onset of the terminal syndrome was seen as markedly reduced feed intake and melena. Over the following nine days, the decreased feed intake progressed to near-complete anorexia and hematochezia appeared. Weight loss, lethargy, hindlimb lameness and weakness were observed. Both dogs had increases in total protein, globulins, phosphorus, cholesterol, triglycerides and fibrinogen. One dog had pancytopenia, decreased hematocrit, hemoglobin, reticulocytes, albumin, and PT and increased bands. Hematuria was also present. The other dog also had decreased lymphocytes, eosinophils, chloride, and sodium and increases in RBC, hematocrit, hemoglobin, platelets, ALP, amylase, creatinine, BUN, magnesium, potassium, and total bilirubin. The clotting profile showed a decreased PT and an increased activated partial thromboplastin time (APTT). These dogs showed lymphoid depletion in lymph nodes, thymus, and gut-associated lymphatic tissues and mild to marked gastrointestinal lesions in addition to the microscopic findings described in animals surviving to the end of the study (see below). These two dogs also had lesions in the gastrointestinal tract, kidneys, pancreas, pituitary gland and adrenal glands.

Unique for these two dogs: While minimal hemosiderosis was present, neither of these dogs showed evidence of extramedullary hematopoiesis in the spleen. Lymph nodes showed moderate to marked nonspecific reactive changes including slight to marked sinus erythrocytes, sinus histiocytosis and plasmacytosis. The female's mesenteric lymph node had a minimal vasculitis of adjacent small vessels.

Glomerular and tubular changes in the kidneys were slight in the male and moderate in the female, with some congestion and hemorrhage in the female. The glomerular changes were mainly characterized by deposition of pale eosinophilic material, only occasionally pink and hyaline, in Bowman's space and sometimes in glomerular tufts. The tubular changes, frequently involving the proximal tubules, were represented by dilatation, with the lumens empty or containing eosinophilic casts and, rarely, red blood cell casts, and by regenerative basophilia. These changes correlated for the female with the severe increases in urea and with the slight increases in creatinine.

1) Clinical Observations and General Health Observations:

Table 17: Number of dogs with the following clinical signs

Clinical Sign	0 mg/kg	2 mg/kg	4 mg/kg	6 mg/kg
Stiffness	0	0	0	1
Weakness – Limb	0	0	0	2
Pain – Limb	1	1	1	3
Lameness	1	1	3	5
Redness of the oral mucosa	1	0	2	5
Skin Ulceration	0	0	0	1
Alopecia	0	1	1	1

All dogs had diarrhea at some point during the study.

2) Body Weight: Dogs in the 4 and 6 mg/kg groups consistently and continually lost weight compared to dogs in the 2 mg/kg group. Dogs in the 4 mg/kg started to have weight loss beginning on Day 31.

3) Food Consumption: Dogs in the 6 mg/kg group had lower food consumption compared to the other groups. The differences in mean daily feed consumption between placebo and the high dose were greatest between Days 21 and 42, ranging from 71 to 98 g per day.

4) Clinical Pathology

Hematology

Red blood cells (RBC): RBC counts, hematocrit, and hemoglobin, showed a treatment-related decrease. Dogs in the 6 mg/kg group had the lowest levels followed by the dogs in the 4 mg/kg group. Two dogs in the 6 mg/kg group were anemic from days 14 to 76 and 40 to 76, respectively. One dog in the 4 mg/kg group was anemic from days 56 to 76.

Reticulocytes: Reticulocyte counts in the 4 and 6 mg/kg groups were significantly lower than placebo on Days 14 and 28, but not thereafter, indicating that after a temporary reduction, production of erythrocytes recovered. In the 6 mg/kg group, 1 dog showed a prominent decrease in RBC count followed by a pronounced reticulocyte response.

Platelets: The mean platelet count increased in a dose dependent manner; however, no treatment group means exceeded the reference range for the laboratory. Dogs in the 4 and 6 mg/kg groups had steadily increasing values up to day 56 which then leveled off. One dog in the 6 mg/kg group had thrombocytosis

from day 21 until the end of the study. One dog in the 6 mg/kg group was thrombocytopenic throughout the study.

Leukocytes (WBC): All treated groups (2, 4 and 6 mg/kg) had leukopenia throughout the study compared to placebo. Dogs in the 6 mg/kg group were panleukopenic. Neutrophils were decreased in a dose dependent manner throughout the study. The majority of dogs in the 4 and 6 mg/kg groups had neutropenia. There was a lack of bands, which implies a lack of bone marrow response.

Basophils and eosinophils were decreased in a dose dependent manner throughout the study. Dogs in the 6 mg/kg group were eosinopenic throughout the study. Mean lymphocyte counts were decreased in a dose dependent manner. Two dogs in the 2 mg/kg group and the majority of dogs in the 4 and 6 mg/kg group had lymphopenia. Monocytes were not affected.

Coagulation Profile: No drug related differences were seen in PT or APTT. Mean fibrinogen concentrations across the study were higher in the 4 mg/kg and 6 mg/kg groups compared to placebo.

Serum Chemistry

Albumin: The mean albumin was consistently lower in the 4 and 6 mg/kg groups compared to the placebo and 2 mg/kg group; however, no group means fell below the lower reference range of the laboratory. One dog in the 6 mg/kg group had hypoalbuminemia throughout the study. Two dogs in the 6 mg/kg group had hypoalbuminemia on day 56.

Globulins: The mean globulin was higher in the 6 mg/kg group compared to other groups. Three dogs in the 6 mg/kg group had elevated globulin levels during the study. One dog in the 2 mg/kg group had persistently elevated globulins and low albumin during the study.

Total Protein (TP): The mean TP was elevated in the 6 mg/kg group compared to the other groups throughout the study. The change in TP consisted of increases in globulin and decreased albumin across the study. One dog in the 2 mg/kg group had an elevated TP throughout the study. One dog in the 4 mg/kg group had an elevated TP on Days 14, 28, 40, and 56. Two dogs in the 6 mg/kg group had rising TP values prior to euthanasia. One dog in the 6 mg/kg group had decreased TP throughout the study.

Alkaline Phosphatase (ALP): Dogs in the 6 mg/kg group consistently had higher levels of ALP throughout the study compared to the other groups but were within the normal reference range. One dog in the 2 mg/kg group had a slightly elevated ALP (less than 3X ULN) during the study.

Aspartate Aminotransferase (AST): The mean AST was elevated (less than 2X ULN) in the 6 mg/kg group compared to the other groups. AST was elevated in 3 dogs in the 6 mg/kg group throughout the study. One dog in the 4 mg/kg group had elevated AST (less than 2X ULN) at most time points.

Amylase: The mean amylase values for the 6 mg/kg group were elevated above the other groups throughout the study. One dog in the 2 mg/kg group had increasing amylase through the study. One dog each in the 4 and 6 mg/kg groups had elevated amylase throughout the study.

Phosphorus: The mean phosphorus was higher in the 4 and 6 mg/kg groups compared to the placebo and 2 mg/kg group. One dog in the 6 mg/kg group was hyperphosphatemic. No group means exceeded the laboratory normal reference range.

Potassium: Serum potassium concentrations in dogs in the 6 mg/kg group were slightly higher than placebo across the study; however, the 6 mg/kg group mean did not exceed the normal reference range of the laboratory.

Sodium: There was a decreasing trend of mean sodium in the 4 and 6 mg/kg groups throughout the study; however, no group means were below the lower reference range of the laboratory.

Creatine Kinase (CK): There was a dose related elevation of CK throughout the study. However, the elevation was within the normal range. The slight increases in CK were correlated with slight increases in LDH.

Lactate Dehydrogenase (LDH): Mean LDH was higher in the 6 mg/kg group throughout the study. Three dogs in the 6 mg/kg group had elevated LDH from Day 40 until the end of the study. One dog in the 4 mg/kg group had elevated LDH on Days 40 and 56 and 1 dog in the 4 mg/kg group had elevated LDH on Days 56 and 70.

5) Pharmacokinetics

C_{max} was shown to be dose proportional in females, but greater than dose proportional in males. There was no significant difference between males and females with respect to the AUC0-t(last), and the AUC0-t(last) was shown to be dose proportional for the duration of the study. Tests of drug accumulation ratios between study days for C_{max} and AUC0-t(last) showed no indication of drug accumulation. There was a significant difference between dose groups in T_{max} by study day and by sex by study day. This was most notable in that the T_{max} occurred marginally later in males through the progression of the study.

6) Pathology

Table 18 below summarizes the findings on histopathologic examination.

Table 18: Histopathologic findings: Number of dogs affected divided by the total number of dogs examined in each group

Organ	0 mg/kg	2 mg/kg	4 mg/kg	6 mg/kg
Bone Marrow				
Reduced Cellularity				
Slight	0	2	3	1
Moderate	0	1	4	2
Marked	0	1	1	1
Total	0/12	4/8	8/12	4/6
Adrenals				
Adrenal Cortical Congestion/Hemorrhage				
Minimal	0/12	3/8	8/12	4/6
Adrenal Cortical Vacuolation				
Minimal	2	0	1	1
Slight	0	1	1	1
Moderate	0	0	1	0
Total	2/12	1/8	3/12	2/6
Pancreas				
Acinar degranulation				
Slight	0	4	5	1
Moderate	0	0	4	5
Total	0/12	4/8	9/12	6/6
Spleen				
Hemosiderosis				
Minimal	3	4	7	0
Slight	2	3	5	6
Total	5/12	7/8	12/12	6/6
Extramedullary hematopoiesis				
Minimal	0	4	7	4
Slight	0	0	3	2
Total	0/12	4/12	10/12	6/6
Testes				
Germ Cell Depletion	1/6	4/4	6/6	3/3
Reduction in spermatozoa	1/6	4/4	6/6	3/3
Ovaries				
Regressed Corpora				

Lutea (CL)	6/6	4/4	5/6	3/3
Absence of CL	0/6	1/4	1/6	0/3
Small follicles	1/6	0/4	5/6	3/3

Bone Marrow
Pale and scant bone marrow was noted in a male in the 4 mg/kg group. Reduced cellularity of sternal and femoral bone marrow, graded as slight to marked, was noted. The severity of the change ranged as slight to marked. The change correlated with the decreases in red and white blood cell counts. A compensatory change, graded as minimal to slight, occurred in the spleen at all doses, consisting of extramedullary hemopoiesis, mainly erythropoiesis. Minimal to slight, dose-related splenic hemosiderosis also was observed, possibly related to minor microhemorrhages recorded in a few tissues.

Pancreas
In the pancreas, dose-related slight to moderate acinar degranulation, characterized by diffuse loss of zymogen granules, occurred in both sexes at all doses. The change was observed in 4 dogs in the 2 mg/kg group, in 9 dogs in the 4 mg/kg group and in all dogs in the 6 mg/kg group.

Lymph node (plasmacytosis)
Dose related plasmacytosis was noted in the mandibular lymph nodes from 1 dog in the 4 mg/kg group and from 3 dogs in the 6 mg/kg group.

Adrenal Glands
In the adrenal glands, minimal cortical congestion/hemorrhage occurred at all doses, with some dose-relationship for the incidence. This change occurred mainly in the outer zona fasciculata and the zona glomerulosa, showed a focal or multifocal distribution, and was characterized by a collection of red blood cells in the sinusoids and only occasionally by microhemorrhages, which were recorded with congestion due to the minimal severity of both changes. Cortical vacuolation was observed at minimal severity in at least 1 dog in all groups, and 1 dog with moderate severity in the 4 mg/kg group, suggesting a possible treatment relationship.

Testes
In males, the testes showed dose-related tubular changes. There was a dose-related depletion of tubular germ cells, marked to severe at the high dose, and corresponding reductions in spermatozoa within the epididymides. Additionally, testicular tubules in some dogs in the 4 and 6 mg/kg groups were affected by a slight to moderate vacuolation. The above changes of the testes correlated with the decreases in mean testes weights recorded on gross examination.

Ovaries
In females, the ovaries were characterized by a reduced incidence of mature/regressing corpora lutea at all doses, without dose-relationship, including individual dogs at 2 and 4 mg/kg doses showing a unilateral or bilateral complete absence of corpora lutea. Increased incidence of small follicles was additionally observed in dogs in the 4 and 6 mg/kg groups, without dose-relationship, when compared with controls.

Relationship of Bone Marrow Suppression and Skin Infections
The focal ulcerations, with acute inflammation and presence of bacteria, noted in the skin from 1 dog in the 4 mg/kg group and in the oral mucosa from 1 dog in the 6 mg/kg group, may indicate an increased susceptibility to local infections consequent to suppressed bone marrow.

g) Conclusion: PHA-291639E has a low margin of safety. Administration of PHA-291639E orally once every other day to Beagle dogs at 0, 2.0, 4.0, and 6.0 mg/kg (0, 0.5, 1, or 1.5 times the clinical dose), for 13 consecutive weeks, caused weight loss, decreased feed consumption, diarrhea, and pancreatic, gonadal, adrenal, muscle-locomotor, and hematopoietic changes including bone marrow suppression. Two dogs at 6 mg/kg were euthanized after 23 and 27 days on treatment with additional signs including progressive anorexia and weakness, lymphoid depletion and gastrointestinal effects.

IV. HUMAN FOOD SAFETY:

This drug is intended for use in dogs, which are non-food animals. Because this new animal drug is not intended for use in food producing animals, CVM did not require data pertaining to drug residues in food (i.e., human food safety) for approval of this NADA.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to PALLADIA:

HUMAN WARNINGS:
NOT FOR USE IN HUMANS. KEEP THIS AND ALL MEDICATIONS OUT OF THE REACH OF CHILDREN. Children should not come in contact with PALLADIA. Keep children away from feces, urine, or vomit of treated dogs.

To avoid exposure to drug, wash hands with soap and water after administering PALLADIA and wear protective gloves to prevent direct contact with feces, urine, vomit, and broken or moistened PALLADIA tablets. Place all waste materials in a plastic bag and seal before general disposal. If eyes are accidentally exposed to the drug, rinse eyes with water immediately.

In case of accidental ingestion by a person, seek medical advice immediately, show the package insert or label to the physician. Gastrointestinal discomfort such as vomiting or diarrhea may occur if this drug is accidentally ingested.

Pregnant women, women who may become pregnant, or nursing mothers should pay special attention to these handling precautions (See handling instructions above.) PALLADIA, like other drugs in its class, prevents the formation of new blood vessels in tumors. In a similar manner, PALLADIA may affect blood vessel formation in the developing fetus and may harm an unborn baby (cause birth defects). For pregnant women, accidental ingestion of PALLADIA may have adverse effects on pregnancy.

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that PALLADIA, when used according to the label, is safe and effective for the treatment of Patnaik grade II or III, recurrent, cutaneous mast cell tumors with or without regional lymph node involvement in dogs.

A. Marketing Status:

This product may be dispensed only by or on the lawful order of a licensed veterinarian (Rx marketing status). Adequate directions for lay use cannot be written because professional expertise is required to properly diagnose mast cell tumors and to monitor the safe use of the product, including treatment of any adverse reactions.

B. Exclusivity:

Under section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of the approval because no active ingredient of the new animal drug has previously been approved.

C. Patent Information:

PALLADIA is under the following U.S. patent numbers:

U.S. Patent Number	Date of Expiration
7,211,600	December 22, 2020
6,573,293	February 15, 2021

For current information on patents, see the Animal Drugs @ FDA database (formerly the Green Book) on the FDA CVM internet website.

VII. ATTACHMENTS:

Facsimile Labeling:
Package Insert
Bottle Label 10 mg
Bottle Label 15 mg
Bottle Label 50 mg